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Pharmacogenomics study in a Taiwanese methadone maintenance cohort

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ABSTRACT

Pharmacogenomics is the study of drug treatment responses in subgroups of patients on the basis of their genetic variants or genetic expression information. In 2006, the government of Taiwan launched a methadone maintenance treatment program, which is usually prescribed for patients with heroin dependence. In this study, 366 patients who had taken methadone continually in the previous 7 days were examined. Data from the Treatment Outcomes Profile (TOP), Severity of Dependence Scale (SDS), Clinical Opioid Withdrawal Scale (COWS), and Treatment Emergent Symptoms Scale (TESS) were obtained from the patients' report records. The genes *CYP2B6*, *CYP3A4*, and *CYP2C19*—which encode the liver cytochrome P-450 (CYP) enzymes that are involved in the metabolism of methadone—were selected and genotyped in this cohort. We found that single nucleotide polymorphisms (SNPs) on the *CYP2B6* gene were associated with the plasma S-methadone concentration. The SNPs on the *CYP3A4* gene were associated with withdrawal symptoms and side effects. The SNPs on the *CYP2C19* gene were associated with the methadone dose. The SNPs in the genes (e.g., *UGT2B7*) encoding the morphine phase II metabolic enzyme were associated with the withdrawal symptom scores. In pharmacodynamic genes, the SNPs on the *OPRM1* gene were associated with the side effects of insomnia and change in libido. We conclude that SNP markers may be useful in the future for adjusting methadone dosage and for reducing adverse reactions.

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1. Introduction

After administering a drug, the steady-state plasma drug concentration may vary from individual to individual. Research reports suggest that the steady-state plasma drug concentrations may change from 5 to 10 times in males with a body weight of approximately 70 kg [1]. If gender, age, and pregnancy status are taken into consideration, the steady-state plasma drug concentrations may vary 50–100 times. Based on this result, it is impossible by using therapeutic drug monitoring to place patients in subgroups to examine treatment responses. Pharmacogenomics attempts to use information about an individual's genomic make-up to place patients in subgroups and match them with a more optimal medication choice. This is a new field of pharmacology. Single nucleotide polymorphism (SNP) is a genomic marker that is often used for subgrouping patients by their treatment responses.

A successful pharmacogenomics clinical trial requires a set criteria. The most important criterion is to ensure the participating patients' compliance during observation by research nurses and during the measurement of the steady-state drug concentrations. A well-designed clinical trial is another key to achieving a successful pharmacogenomics study.

In 2006, the Taiwanese government established a methadone maintenance treatment (MMT) program. Since then, more than 90 hospitals have provided MMT and more than 11,000 heroin-dependent patients have participated in this program. The purpose of this program is to reduce heroin abuse, reduce the spread of infectious diseases such as human immunodeficiency virus (HIV) infection and hepatitis C virus (HCV) infection, and reduce the drug-related crime rate. Because individual methadone doses range from 5 mg/day to 180 mg/day, a drug–drug interaction may cause an overdose and may induce lethal complications. Therefore, the National Health Research Institutes (NHRI; Miaoli County, Taiwan) launched a pharmacogenomics study in a MMT cohort.

2. Methods

2.1. Study participants

This study was a cross-sectional methadone clinical trial. The study was approved by the institutional review boards of the National Health Research Institutes (Miaoli County, Taiwan) and the following seven participating hospitals: Tao-Yuan Mental Hospital (Taoyuan City, Taiwan); En-Chu-Kong Hospital (New Taipei City, Taiwan); Far Eastern Memorial Hospital (New Taipei City, Taiwan); Taipei City Hospital, Song-De Branch (Taipei, Taiwan) and Yang-Ming Branch (Taipei, Taiwan); China Medical University Hospital (Taichung City, Taiwan); and Wei Gong Memorial Hospital (Toufen, Taiwan). Each study participant provided written, informed consent. The project also was registered in the U.S. National Institutes of Health Clinical Trial database (http://www.clinicaltrial.gov/ct/show/NCT01059747).

We recruited 366 heroin-dependent individuals who were undergoing MMT as outpatients. The inclusion criteria for participants were: aged 18 years or older, being under MMT for at least 3 months with regular attendance in the previous 7 days, and a methadone dosage adjustment of no more than 10 mg in the previous 7 days. The exclusion criteria were: comorbidity with other physical or mental disorders that require immediate treatment or pregnancy.

2.2. Clinical assessments

The demographics, clinical characteristics, and methadone treatment courses (e.g., dose, treatment duration, and treatment adherence) for the previous week were obtained from the patients' report records [e.g., case report form (CRF)]. Information regarding the possible coadministration of other medications in the previous week was obtained either from the patients' medical records or from the patients' reports [2]. Prior to when methadone was administered, each patient underwent several interviewer-administered assessments such as: the Treatment Outcomes Profile (TOP), which measures the amount and frequency of alcohol and other illicit substances used in the past 28 days [3]; the Severity of Dependence Scale (SDS); and the Clinical Opioid Withdrawal Scale (COWS), which measures the severity of 11 opioid withdrawal symptoms [4].

Research nurses assessed methadone-related adverse events by using the Treatment Emergent Symptoms Scale (TESS), which consists of 43 treatment emergent symptoms [5]. In this study, the patients only reported 31 symptoms. Only symptoms that occurred after the initiation of MMT were counted as methadone-related adverse events. The severity of each symptom was rated on a three-point Likert scale ranging from mild (one point), moderate (two points), to severe (three points).In the present study, only adverse events with a frequency of 15% or higher were included in the analyses.

2.3. Serum and urine drug test

The levels of glutamate oxaloacetate transaminase (GOT; reference range, less than 38 U/L), glutamic pyruvic transaminase (GPT; reference range, less than 41 U/L), and gamma-glutamyl transpeptidase (γ-GT; reference range, 8–61 U/L) from serum samples of patients were measured at the Taipei Institute of Pathology (Taipei, Taiwan).

Urine specimens were collected prior to the administration of methadone on the study day. A morphine screen test was performed via a kinetic interaction of microparticles on the Integra 800 device (Roche Diagnostics, Basel, Switzerland). In our present analyses and in previous reports [2,6,7], the urine morphine test was used as a surrogate measurement for the methadone treatment outcome.

2.4. Analyses of methadone and its metabolites in the plasma

Whole blood samples (12 mL) were obtained at approximately 24 ± 2 h after the last methadone dose, when the plasma concentration of methadone is likely to be at its lowest. The plasma concentrations of racemic methadone and its metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), were measured by using high-performance liquid chromatography (HPLC) at the settings described in our previous study [8].

Table 1 – General demographics of the 366 methadone maintenance treatment patients.

	Overall	Urine morphine positive	Urine morphine negative	*p*
	N = 366	*n* = 185	*n* = 178	
	Mean ± SD	Mean ± SD	Mean ± SD	
Starting dose of methadone (mg/d)	32.04 ± 11.15	31.64 ± 10.07	32.44 ± 12.22	0.77*
Current dose of methadone (mg/d)	54.67 ± 28.12	54.53 ± 26.07	55.32 ± 30.13	0.88*
Age (y)	38.17 ± 7.72	38.37 ± 7.96	37.87 ± 7.46	0.57*
Male	297 (81.2%)	152 (82.2%)	142 (79.8%)	0.56**
BMI (kg/m^2)	23.58 ± 3.52	23.62 ± 3.57	23.59 ± 3.49	0.89*
R-methadone concentration/methadone dose ratio	3.86 ± 2.32	3.7 ± 2.71	4.03 ± 1.82	**0.001***,***
S-methadone concentration/methadone dose ratio	2.77 ± 1.57	2.58 ± 1.45	2.98 ± 1.66	**0.012***,***
R-EDDP concentration/methadone dose ratio	0.31 ± 0.5	0.26 ± 0.33	0.33 ± 0.54	0.26*
S-EDDP concentration/methadone dose ratio	0.33 ± 0.49	0.31 ± 0.38	0.33 ± 0.58	0.95*
Human immunodeficiency virus infection	86 (24.0%)	50 (27.3%)	36 (20.8%)	0.15**
Hepatitis C virus infection	334 (94.9%)	173 (96.1%)	158 (93.5%)	0.27**

Data are presented as mean ± SD or *n* (%).
* The *p* value is based on the Wilcoxon rank-sum test.
** The *p* value is based on the Chi-square test.
*** A *p* value < 0.05 is presented in bold font.
BMI = body mass index; EDDP = 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine.

2.5. *Single nucleotide polymorphism selection and genotyping*

The selection of SNPs was based on reports in the literature [2,6,9–13], a minor allele frequency greater than 0.1 on the HapMap in Chinese ethnic groups (http://hapmap.ncbi.nlm.nih.gov/index.html.en), and possible functionalities predicted by the bioinformatics tool FastSNP. This is not a kit. It is a paper and also had an web-page for bioinformatics analyses [14].

Genomic DNA was extracted from the buffy coat of whole blood lymphocyte pellets (6 mL) by using the Puregene Blood Kit C (QIAGEN Sciences, Maryland, USA). Genotypes of the selected 17 SNPs were identified by using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) [15]. This genotyping method has been utilized in a broad variety of clinical applications because of the accuracy of SNP detection, the sufficient sensitivity to score SNPs from small amounts of template, the flexibility of the procedure, and the cost effectiveness [16].

2.6. *Statistics*

All statistical analyses were conducted by using SAS version 9.1 software (SAS Institute, Inc., Cary, NC, USA). The Wilcoxon rank-sum test (for continuous data) and the Chi-square test or Fisher's exact test (for categorical data) were applied for comparing the clinical characteristics, the TESS scores, and the COWS scores of patients testing positive for urine morphine and patients testing negative for urine morphine. Association analyses were calculated by using a generalized linear model (GLM) procedure to compare the selected SNPs with each of the following: treatment outcomes, withdrawal symptoms, and adverse events. The false discovery rate test of the MULTTEST procedure was employed to correct for multiple hypotheses tests of *p* values [17]. The Hardy-Weinberg equilibrium test and haplotype block association analysis were performed by using HAPLOVIEW version 4.1 software (Cambridge, MA, USA) [18]. A statistical significance was a $p < 0.05$.

3. Results

3.1. *General clinical characteristics*

One-half of the 366 MMT patients tested positive for urine morphine (Table 1). The average age of these patients was 38.2 ± 7.7 years and nearly all (99.5%) participants smoked cigarettes. Males were predominant in this cohort (*n* = 297; 81.1%). The average starting dose of methadone was 32 ± 11 mg/day, and the dosage at the time of testing was 54.7 ± 28 mg/day. The ratio of plasma R-methadone concentration/methadone dose and the ratio of plasma S-methadone concentration/methadone dose were higher in patients who tested negative for urine morphine than in patients who tested positive. The HCV incidence in these patients was 95%. In body mass index and in test results for HIV and HCV infection, there were no significant differences between patients who tested positive for urine morphine and patients who tested negative for urine morphine.

The average COWS withdrawal symptom score was 1.49 ± 1.86 points (Table 2). There was a statistically significant higher incidence of bone and joint aches symptom scores in patients testing positive for urine morphine (13% incidence and 0.14 average score) than for patients who tested negative for urine morphine (6.2% incidence and 0.08 average score). Table 3 lists the top 15 side effects (based on percentage), as rated by TESS. The side effect of impaired mentation showed a significantly higher incidence in patients who tested positive for urine morphine than in patients who tested negative (22.2% vs. 20.8%, respectively).

3.2. *Association analyses of pharmacokinetic genes and methadone dose*

Methadone is primarily metabolized in the liver through specific isoforms of the cytochrome P-450 (CYP) enzyme system [19]. These isoforms include *CYP2B6*, *CYP2C19*, and

Table 2 – Withdrawal symptoms of the 366 methadone maintenance patients.

	Overall			Urine Morphine Positive			Urine Morphine Negative			*p*	*p*
	N = 366			N = 185			N = 178				
	n	%	Mean ± SD	*n*	%	Mean ± SD	*n*	%	Mean ± SD		
Sum of the COWS score	366		1.49 ± 1.86	185		1.51 ± 1.93	178		1.44 ± 1.78	0.85*	
Heart rate	365		77.55 ± 11.86	184		76.83 ± 11.29	178		78.20 ± 12.24	0.37*	
Sweating	41	11.20	0.13 ± 0.40	26	14.10	0.17 ± 0.48	14	7.90	0.08 ± 0.30	0.06*	0.06**
Restlessness	24	6.60	0.07 ± 0.25	15	8.10	0.08 ± 0.27	8	4.50	0.04 ± 0.21	0.16*	0.16**
Pupil size	69	18.90	0.21 ± 0.46	34	18.50	0.20 ± 0.44	35	19.70	0.22 ± 0.48	0.74*	0.77**
Bone or joint aches	36	9.80	0.11 ± 0.36	24	13.00	0.14 ± 0.38	11	6.20	0.08 ± 0.33	**0.033****	**0.028**,****
Runny nose or tearing	31	8.50	0.11 ± 0.39	14	7.60	0.10 ± 0.36	17	9.60	0.13 ± 0.43	0.49*	0.50**
Gastrointestinal upset	16	4.40	0.07 ± 0.33	9	4.90	0.08 ± 0.39	7	3.90	0.05 ± 0.27	0.65*	0.67**
Tremor	39	10.70	0.15 ± 0.46	20	10.80	0.13 ± 0.40	19	10.70	0.17 ± 0.53	0.91*	0.97**
Yawning	10	2.70	0.04 ± 0.23	6	3.20	0.04 ± 0.25	3	1.70	0.02 ± 0.18	0.34*	0.50****
Anxiety or irritability	39	10.70	0.12 ± 0.35	19	10.30	0.12 ± 0.37	19	10.70	0.11 ± 0.31	0.94*	0.90**
Gooseflesh skin	8	2.20	0.07 ± 0.44	4	2.20	0.06 ± 0.44	4	2.30	0.07 ± 0.45	0.96*	1.00****

COWS = Clinical Opiate Withdrawal Scale.
* The *p* value is based on the Wilcoxon rank-sum test.
** The *p* value is based on the Chi-square test.
*** A *p* value < 0.05 is presented in bold font.
**** The *p* value is based on Fisher's exact test.

CYP3A4, which have metabolic preferences toward the two different methadone enantiomers (Fig. 1). The *R*-form enantiomer is preferably metabolized by *CYP2C19* and the *S*-form enantiomer is preferably metabolized by *CYP2B6* [19]. In our previous studies, the SNPs on *CYP2B6* were significantly associated with the plasma *S*-methadone concentration, the *S*-methadone concentration/methadone dose ratio, and the *S*-methadone clearance [6]. We also found that *CYP2B6* has a higher level of activity in HCV-positive patients. The SNPs in *CYP3A4* were significantly associated with the COWS score, TESS score, and betel nut use [12]. As the withdrawal symptom scores increased, the side effect symptom score increased, but betel nut use decreased. The gene dose of *CYP2C19* showed significant correlations with methadone dose, *R*-methadone concentration/methadone dose ratio, and *R*-EDDP concentration/methadone dose ratio [13]. The gene dose was associated with the TESS scores in patients testing positive for urine morphine. Patients who were extensive metabolizers had a higher side effect score than patients who were poor metabolizers.

The gene–gene interaction association analyses indicated that the *CYP2C19* gene may not interact with *CYP2B6*

Table 3 – The top 15 side effects of the 366 methadone maintenance patients.

	Overall			Urine Morphine Positive			Urine Morphine Negative			*p* *	*p* **
	N = 366			N = 185			N = 178				
	n	%	Mean ± SD	*n*	%	Mean ± SD	*n*	%	Mean ± SD		
Constipation	248	67.80	1.96 ± 0.84	125	67.60	2.01 ± 0.85	121	68.00	1.92 ± 0.83	0.4	0.93
Sedation	172	47.00	1.51 ± 0.70	94	50.80	1.51 ± 0.68	77	43.30	1.49 ± 0.72	0.73	0.15
Change in libido	111	30.30	1.72 ± 0.79	63	34.10	1.70 ± 0.75	47	26.40	1.77 ± 0.84	0.76	0.11
Dry mouth	101	27.60	1.55 ± 0.70	59	31.90	1.61 ± 0.74	41	23.00	1.49 ± 0.64	0.49	0.06
Excessive sweating	71	19.40	1.86 ± 0.85	38	20.50	1.95 ± 0.87	32	18.00	1.75 ± 0.84	0.34	0.54
Insomnia	67	18.30	1.93 ± 0.78	32	17.30	2.03 ± 0.78	34	19.10	1.85 ± 0.78	0.36	0.66
Impaired mentation	79	21.60	1.61 ± 0.72	41	22.20	1.78 ± 0.76	37	20.80	1.43 ± 0.65	**0.032****	0.75
Fatigue	65	17.80	1.62 ± 0.78	32	17.30	1.78 ± 0.79	32	18.00	1.47 ± 0.76	0.08	0.87
Difficulty with urination	52	14.20	1.38 ± 0.60	28	15.10	1.50 ± 0.69	23	12.90	1.26 ± 0.45	0.24	0.54
Increase in appetite	46	12.60	1.48 ± 0.59	25	13.50	1.52 ± 0.51	21	11.80	1.43 ± 0.68	0.37	0.62
Decrease in appetite	44	12.00	1.55 ± 0.70	26	14.10	1.50 ± 0.71	18	10.10	1.61 ± 0.70	0.54	0.25
Weight gain	38	10.40	1.68 ± 0.74	18	9.70	1.67 ± 0.59	20	11.20	1.70 ± 0.86	0.86	0.64
Weakness	33	9.00	1.36 ± 0.70	18	9.70	1.56 ± 0.86	14	7.90	1.14 ± 0.36	0.17	0.53
Malaise	27	7.40	1.48 ± 0.64	13	7.00	1.62 ± 0.65	13	7.30	1.38 ± 0.65	0.31	0.92
Tachycardia/palpitations	25	6.80	1.44 ± 0.71	14	7.60	1.43 ± 0.65	10	5.60	1.50 ± 0.85	1.00	0.45

* The *p* value is based on the Wilcoxon rank-sum test.
** The *p* value is based on the Chi-square test.
***A *p* value < 0.05 is presented in bold font.

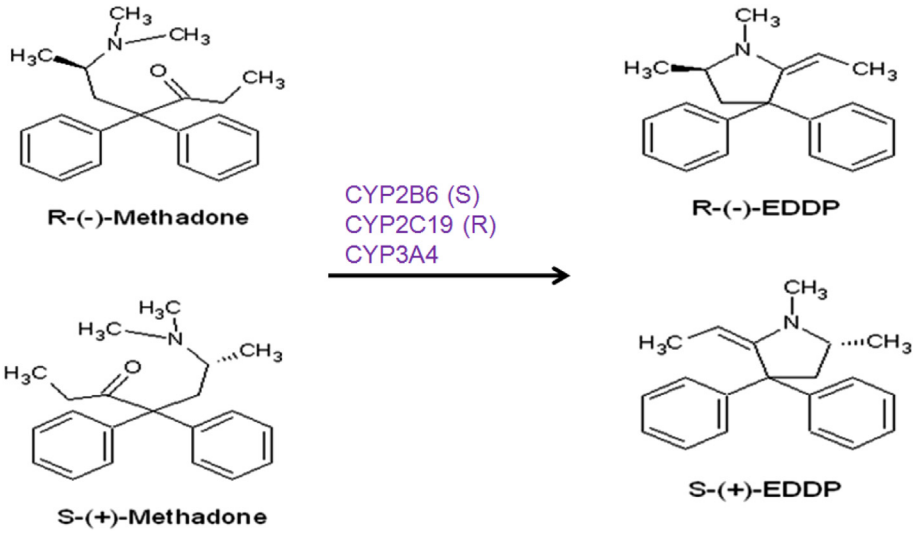

Fig. 1 – The methadone metabolic pathway. EEDP = 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine.

or *CYP2C19*. However, the allele types in *CYP2B6* or *CYP3A4* may allow the subgrouping of the methadone dose into six dosage ranges and may increase the level of significant associations between the gene dose of *CYP2C19* and the methadone dose. In three-gene interaction association analyses, *CYP2C19* did not interact with either *CYP2B6* or *CYP3A4*. The allelic combination of *CYP2B6* and *CYP3A4* further subgrouped the methadone dose into 12 dose ranges and increased the level of significant association between *CYP2C19* and the methadone dose from $p = 0.018$ to $p < 10^{-4}$. This *CYP2C19* interaction was also significantly associated with methadone tolerance when defining it as the dose difference between the current dose and the starting dose of methadone.

In the phase II morphine metabolic enzyme UDP glucuronosyltransferase 2 family, polypeptide B7 (*UGT2B7*), the SNPs in this coding region showed significant associations with the severity of withdrawal symptom, as rated by the COWS scores [7].

3.3. *Association analyses of the pharmacodynamic gene OPRM1*

The pharmacodynamic gene, *OPRM1*, was reported first in the *mu*-opioid receptors. The nonsynonymous *OPRM1* SNP rs1799971 (exon 1, A118G polymorphism, Asn40Asp) showed a marginally significant association with the methadone dose (adjusted GLM; $P = 0.027$). The intron 1 region of the SNPs demonstrated significant associations with the side effects of insomnia and change in libido [2]. It was also significantly associated with the plasma nicotine metabolite cotinine [20]. In the recessive model of association analyses, the patients who had a higher plasma cotinine level had a lower insomnia symptom score. The pharmacokinetic genes may be correlated with physical dependence, whereas the pharmacodynamic genes are correlated to psychological dependence (Fig. 2).

4. Discussion

In this methadone maintenance cohort, we found that it is impossible to explain heroin dependence by using a single gene association analysis in pharmacogenomics studies. The metabolism of methadone involves more than one single cytochrome *P*-450 (CYP) enzyme (e.g., CYP2B6, CYP2C19, and CYP3A4). The genetic variants of each CYP isozyme may play different roles. The patients in this cohort were exposed to multiple environmental influences (e.g., 95% patients tested positive for the HCV antibody). All patients reported smoking cigarettes. It is impossible to report these important environmental influences with a single genetic association analyses.

In the current study, especially in the pharmacokinetic genes—the interaction between *CYP2C19*, *CYP2B6*, and *CYP3A4* may classify the patients into different methadone dosage subgroups with a *p* value lower than the *p* value of the pharmacodynamic *OPRM1* single gene. This result indicated that the required methadone dosage is regulated by genes acting in combination with pharmacokinetic and pharmacodynamic influences. The SNPs in the pharmacokinetic genes (e.g., *CYP2B6*, *CYP2C19*, and *CYP3A4*) may be used as indicators for the plasma methadone concentrations, the methadone dose, and the severity of withdrawal symptoms.

The SNPs in the pharmacodynamic genes may influence the dosage prediction and the treatment side effects. The SNPs in the pharmacodynamic genes, especially the *OPRM1* gene, were associated with the methadone dose and may be predictors for methadone side effects such as insomnia and a change in libido.

Pharmacogenomics studies promise the advent of personalized medicine. In the association analyses of SNPs in single genes, we found that each single gene covers a different number of exons and introns. Using single genetic variants may provide further information for genetic regulation and for

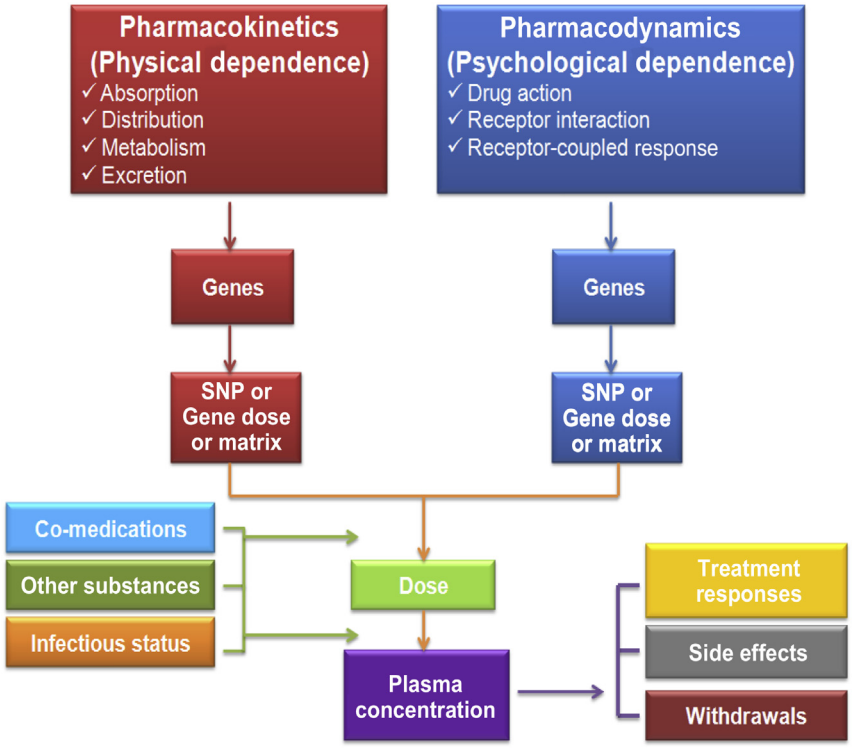

Fig. 2 – Pharmacogenomics study diagram for the methadone maintenance treatment patients.

the functional correlation with responses, which may then be used to direct further biological study or be replicated in the study of other ethnic groups.

The limitations of this cohort include a gender imbalance (81% of the participants were male), a high prevalence of HCV infection (95% of the participants were HCV positive), positive results for urine morphine (approximately 50% of patients tested positive), and the relatively small sample. Replication studies are needed to establish future treatment guidelines.

Additional methods of optimizing drug therapy (with respect to a patient's genotype) warrant further investigation to ensure maximum efficacy with minimal adverse effects. This study took into consideration the potential of using a single pharmacokinetics gene (e.g., *CYP2B6*, *CYP3A4*, *CYP2C19*, *UGT2B7*, and *PXR*) and pharmacodynamic genes (e.g., *OPRM1*). Studying random combinations of these genes and making association analyses without having the results of each single gene would not reveal the individual contributions of each single gene in the systemic methadone treatment response. Gene–gene interaction should be based on single gene discoveries, and not based on a nebulous theory. Because pharmacogenomics studies are still at an early stage, it is essential to study numerous SNP markers in each single gene, which may represent the function of a gene. We therefore showed the SNP IDs so that it would be easier for researchers who are interested in replicating the study with other ethnic groups.

Methadone pharmacogenomics studies may decipher the mechanisms of opioid dependence. There is great potential for pharmacogenomics to be used as a clinical practice guideline for future individualized medicine. Our studies provide essential information in bridging the basic biological functional study through understanding the exon and intron roles of each gene, and the potential drug targeting of novel treatment processes through the association analyses of treatment responses.

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